IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Charles T. Esmon and Naomi L. Esmon

SERIAL NO: 07/730,040 ART UNIT: 182

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FOR: MONOCLONAL ANTIBODY AGAINST PROTEIN C

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

I, Joseph Paul Miletich, Jr. hereby declare that:

1. I am Associate Professor of Medicine in Pathology at Washington University School of Medicine. I received my M.D. and Ph.D. degrees from Washington University in 1979. I have conducted research in the areas of hemostasis and thrombosis.

2. In collaboration with my colleagues at the Washington University School of Medicine we have isolated murine monoclonal antibodies to fifteen different proteins including human prothrombin, human factor V, human factor VII, human factor VIII, human factor IX, human factor X, human factor XIII, human von Willebrand factor, human protein S, human protein Z, human protein C, human tissue factor pathway inhibitor, rabbit prothrombin, rabbit factor VII, and rabbit protein C. Altogether we have immunized mice and fused spleen cells on twelve occasions in which the entire human protein C molecule or parts of the molecule were used as antigens. We used very stringent selection criteria for hybridomas and have identified only ten as positive.

These ten have resulted in six stable cell lines producing useful monoclonal antibodies to human protein C. None of these monoclonal antibodies bind calcium (either with or without the presence of their epitopes), one monoclonal recognizes zymogen but does not recognize activated protein C, and none have precisely the same epitope as HPC-4. Antibodies that show divalent metal cation dependence for binding to their antigen are extremely useful and our screening assays were particularly designed to discover any hybridomas which might show metal ion dependence. Of the thirty or more monoclonals directed against the other proteins mentioned above, only three have shown a calcium dependence of binding to their epitope (one monoclonal that recognizes human protein S, one that recognizes human factor X, and one that recognizes human factor VII). None of these monoclonals show any crossreactivity against protein C.

Based on my eleven years of experience in this field, it is my opinion that it would be extremely difficult to isolate the same hybridoma/antibody as HPC-4, even after reviewing the Sterns, et al. Journal of Biological Chemistry 263:826, 1988 and the Esmon et al. 1987 publication.

3. I declare that all statements made herein of my own knowledge are true. These statements are made with the knowledge that willfull false statements are punishable by fine or imprisonment under section 1001 of Title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Joseph P. Miletich

Joseph P. Miletich, M.D., Ph.D.

Date: 2/24/92